CLAIMS

1. A conjugate comprising an erythropoietin glycoprotein having an N-terminal α-amino group and one poly(ethyleneglycol), said erythropoietin glycoprotein being selected from the group consisting of human erythropoietin, analogs thereof that have from 1 to 6 additional sites for glycosylation, and human erythropoietin having at least one glycosylation site that is rearranged, and being covalently linked to one poly(ethylene glycol) group of the formula

$$-CO-(CH_2)_x-(OCH_2CH_2)_m-OR,$$

wherein the -CO of the poly(ethylene glycol) group forms an amide bond with the N-terminal α-amino group of the erythropoietin glycoprotein;
R is lower alkyl;
x is 2 or 3; and
m is from about 450 to about 1350.

2. The conjugate of claim 1, having the formula

$$P-NHCO-(CH_2)_x-(OCH_2CH_2)_m-OR \qquad (I)$$

wherein P is the residue of the erythropoietin glycoprotein without the N-terminal α-amino group which forms an amide linkage with the poly(ethylene glycol) group.

3. The conjugate of claim 1, wherein R is methyl.

4. The conjugate of claim 1, wherein m is from about 550 to about 1000.

5. The conjugate of claim 4, wherein m is from about 650 to about 750.

6. The conjugate of claim 4, wherein R is methyl.

7. The conjugate of claim 2 having the formula

$$CH_3O(CH_2CH_2O)_mCH_2CH_2CH_2CO\text{-}NH\text{-}P$$

wherein m is from about 650 to about 750.

8. The conjugate of claim 1, wherein the glycoprotein is a human erythropoietin.

9. The conjugate of claim 8, wherein the human erythropoietin glycoprotein is expressed by endogenous gene activation.

10. The conjugate according to claim 8, wherein the glycoprotein has the sequence shown in Fig. 1 or Fig. 2.

11. The conjugate according to claim 8, wherein the glycoprotein has the sequence of human erythropoietin modified by the addition of from 1 to 6 glycosylation sites.

12. The conjugate according to claim 11, wherein the glycoprotein has the sequence of human erythropoietin which is modified by a modification selected from the group consisting of:

$Asn^{30}Thr^{32}$;
$Asn^{51}Thr^{53}$,
$Asn^{57}Thr^{59}$;
Asn^{69};
$Asn^{69}Thr^{71}$;
$Ser^{68}Asn^{69}Thr^{71}$;
$Val^{87}Asn^{88}Thr^{90}$;
$Ser^{87}Asn^{88}Thr^{90}$;
$Ser^{87}Asn^{88}Gly^{89}Thr^{90}$;
$Ser^{87}Asn^{88}Thr^{90}Thr^{92}$;
$Ser^{87}Asn^{88}Thr^{90}Ala^{162}$;
$Asn^{69}Thr^{71}Ser^{87}Asn^{88}Thr^{90}$;
$Asn^{30}Thr^{32}Val^{87}Asn^{88}Thr^{90}$;
$Asn^{89}Ile^{90}Thr^{91}$;

$Ser^{87}Asn^{89}Ile^{90}Thr^{91}$;
$Asn^{136}Thr^{138}$;
$Asn^{138}Thr^{140}$;
Thr^{125}; and
$Pro^{124}Thr^{125}$.

13. The conjugate according to claim 1 , wherein the glycoprotein has the sequence of human erythropoietin modified by a rearrangement of at least one glycosylation site.

14. The conjugate of claim 13, wherein the rearrangement comprises deletion of any of the N-linked glycosylation sites in human erythropoietin and addition of an N-linked glycosylation site at position 88 of the sequence of human erythropoietin.

15. The conjugate of claim 14, wherein the glycoprotein has the sequence of human erythropoietin modified by a modification selected from the group consisting of:
Gln^{24} Ser^{87} Asn^{88} Thr^{90};
Gln^{38} Ser^{87} Asn^{88} Thr^{90}; and
Gln^{83} Ser^{87} Asn^{88} Thr^{90}.

16. A pharmaceutical composition comprising the conjugate of claim 1 and a pharmaceutically acceptable excipient.

17. A method of treating anemia in a patient afflicted with chronic renal failure (CRF) or AIDS or resulting from chemotherapy, comprising administering to said patient an effective amount of a conjugate of claim 1.

18. A process of making a conjugate of claim 1 , comprising

a) expressing and fermenting a recombinant EPO protein that has an N-terminal peptidic extension that includes a proteolytic cleavage sequence,

b) protecting the ε-amino groups,

c) proteolytically cleaving the N-terminal peptidic extension,

d) pegylating the N-terminal α-amino group, and

e) deprotecting the ε-amino groups of the EPO glycoprotein.

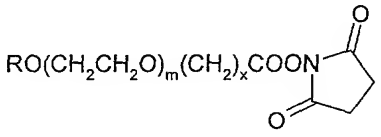
19. The process of claim 18 wherein the fermentation in step a) is serum free.

20. The process of claim 18 wherein any one of steps a)-e) is followed by a purification step.

21. The process of claim 18 wherein the recombinant EPO comprises a sequence selected from the group consisting of the amino acid sequences shown in any one of Figures 1, 2, 3, 4 and 5.

22. The process according to claim 18 wherein in step b) the ε-amino groups are protected by citraconylation.

23. The process of claim 18 wherein the N-terminal α-amino group in step d) is pegylated with a group



(II)

wherein
R is lower alkyl;
x is 2 or 3; and
m is from about 450 to about 1350.

24. An erythropoietin glycoprotein comprising the amino acid sequence of Fig. 1 or Fig. 2 and having an N-terminal peptidic extension that is a proteolytic cleavage site.

25. The erythropoietin glycoprotein of claim 24 which also comprises an N-terminal purification tag.